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Original

Effect of UV Photofunctionalization of HA/TiO_2 Coated Implants Prepared by Dual-Target Sputtering on Bone-Implant Integration

Nobutake Monai[1], Atsushi Kuwabara[1], Norishige Kawanishi[1], Ryotaro Ozawa[1], Takuya Adachi[1], Saki Tsunoi[1], Makoto Inoue[1], Makiko Saita[1], Tohru Hayakawa[2], Noriyuki Hoshi[1,3] and Katsuhiko Kimoto[1]

[1] *Department of Fixed Prosthodontics, Kanagawa Dental University, Kanagawa, Japan*
[2] *Department of Dental Engineering, Tsurumi University School of Dental Medicine, Kanagawa, Japan*
[3] *Department of Education Planning, Kanagawa Dental University, Kanagawa, Japan*

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Abstract: The purpose of this study was to examine the biological response to a hydroxyapatite (HA)/titanium dioxide (TiO_2) hybrid (Hyb) coated implant surface after ultraviolet UV irradiation. After acid etching, titanium (Ti) disc and implant surfaces were modified using HA and TiO_2 targets, employing single-target and dual-target sputter deposition techniques, and subjected to ultraviolet (UV) irradiation. The surface roughness and hydrophilicity were analyzed, and the biomechanical strength of the bone–implant interface was assessed using an implant biomechanical push-in test. We found that UV irradiation improved the strength of the bone-implant interface for all modified Ti surfaces. The Hyb surface showed greater bone-implant integration than the microrough (acid etched) or single-target sputtered surfaces (TiO_2 and HA).

Key words: Dual sputtering deposition, Hydroxyapatite, Titanium dioxide, Photofunctionalization, Bone-implant integration

Introduction

Dental implant therapy is a widely applied recovery treatment for defective prostheses that greatly improves the satisfaction, masticatory ability, and quality of life (QOL) of patients[1,2]. However, dental implant failure rates of 8-15% have been reported due to the increase in use[3,4]. A cause of this high failure rate is poor bone-implant integration after implant surgery[5,6].

To increase the long-term clinical success rate of dental implants, the development of the surface characteristics of the implant as bioactive material has been considered. Numerous studies have developed various effective implant surface treatments, including sandblasting, acid etching, anodic oxidation, functional peptides, fluoride treatment, titanium dioxide (TiO_2) nanonodular structures, hydroxyapatite (HA), and other calcium coatings[7-9]. The treatment involving the HA coating of titanium (Ti) implant surfaces is based on the fact that HA is the main component of bone, HA ($Ca_{10}(PO_4)_6(OH)_2$) is still the most common coating for Ti implant surfaces[10]. It has also been reported that modifying implant surface roughness by coating it with TiO_2 micro-nano particles increases the strength of bone-implant interface and promotes cell adhesion and proliferation[11].

Existing dual-target sputter deposition technology has been applied to combine HA and TiO_2 to produce a HA/TiO_2 hybrid (Hyb) layer for coating on various biomedical devices and implant surfaces[12-14]. This Hyb surface promotes osteoblast adhesion and proliferation in vitro, improves the biomechanical strength of the bone-implant interface in vivo, and generally performs better than single-target sputtered coatings[14].

UV photofunctionalization has been used to modify Ti implant surfaces as well. This technology, involving UV irradiation at the compound wavelength, increases the osteoplastic properties of the Ti surfaces by enhancing the hydrophilicity of the Ti surface and removing the accumulated hydrocarbons[15-18], and has already been clinically applied to dental implant therapy[19,20]. However, the effect of UV photofunctionalization of Hyb surfaces containing both bioactive and bioinert substances is still unknown. The purpose of this study was to investigate biological responses to UV-irradiated Hyb surfaces manufactured using dual-target sputter deposition.

Materials and Methods

Ti substrate and sputter deposition

Ti discs (diameter = 20 mm, thickness = 1.0 mm) and pure Ti cylindrical implants (diameter = 1.0 mm, length = 2.0 mm) are made of grade 2 commercial pure Ti. All Ti samples were treated with 66% sulfuric acid at 120°C for 75 s to create an overall fine rough surface (control). Both samples were then rinsed with pure water. As established[14], to prepare single-target sputtered coatings of HA and TiO_2, respectively, deposition was performed using a target supply of 100 W (HA or TiO_2). Dual target of HA target and TiO_2 target on the clean Ti surface was sputter-deposited by a high frequency magnetron sputtering system (HSD-542, Shimadzu Corporation, Kyoto, Japan). The Hyb layer was controlled by a simultaneous supply of 100 W of TiO_2 sputtering and 200 W of HA sputtering[14]. Film thickness was restricted to 100 nm ± 10% for all substrates.

Photofunctionalization by UV irradiation

The prepared Ti disks and cylindrical implants were irradiated with ultraviolet (UV) rays at the compound wavelength in a photodevice (TheraBeam Affiny, USHIO Inc., Tokyo, Japan). Each Ti surface was exposed to approximately 8–10 mW/cm^2 of radiation, at wavelengths of

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Correspondence to: Dr. Noriyuki Hoshi, Department of education planning & Department of Fixed Prosthodontics, Kanagawa Dental University, 82 Inaoka-cho, Yokosuka, Kanagawa, 238-8580, Japan; Tel: +81-46-822-9503; Fax: +81-46-822-9532; E-mail: hoshi@kdu.ac.jp

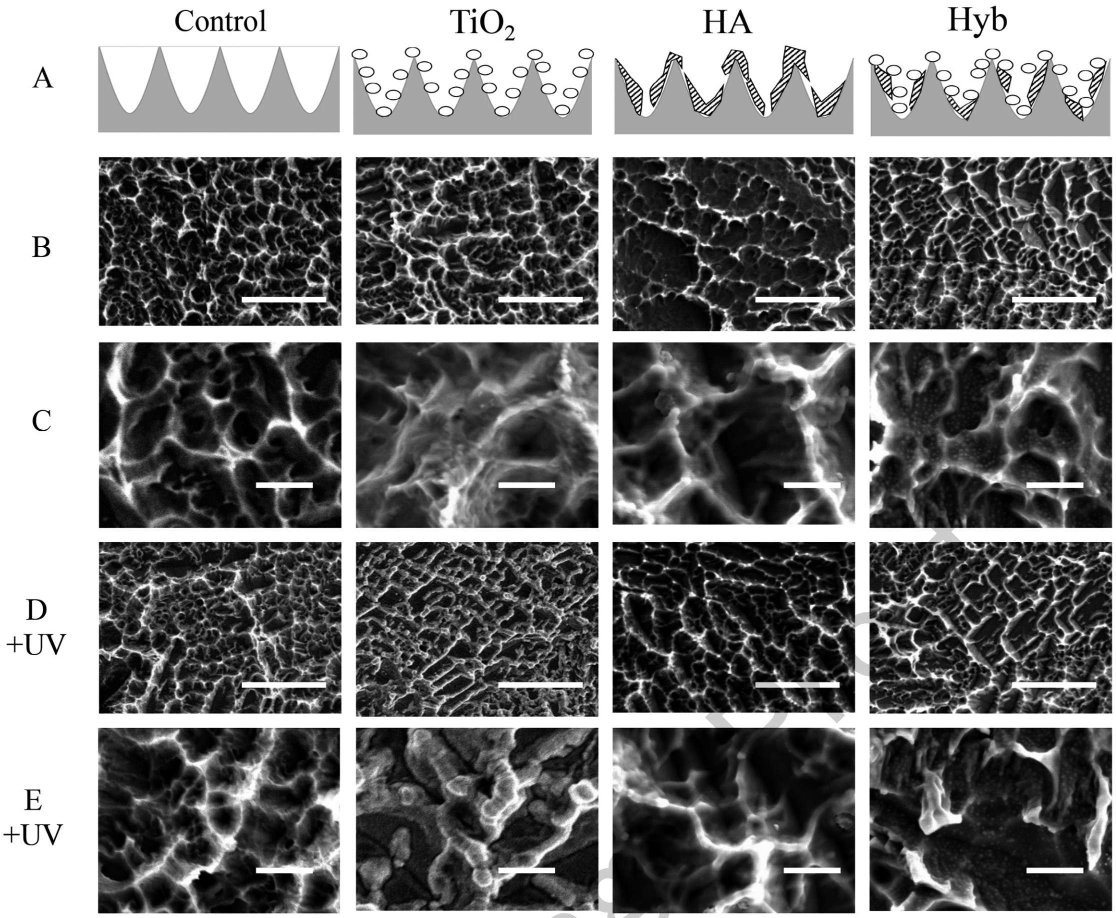

Figure 1. Scanning electron micrographs of the microrough surface and three types of sputter-deposited surfaces. (A): Schematic representation of surface structures. Control: no sputter deposition. TiO$_2$: single-target sputter deposition using Ti dioxide target. HA: single-target sputter deposition using hydroxyapatite target. Hyb: dual-target sputter deposition using both HA and TiO$_2$ targets. ○ : TiO$_2$, ▨ : HA. (B): UV-untreated surface (scale bar = 5 µm). (C): UV-untreated surface (scale bar = 1 µm). (D): UV-treated surface (scale bar = 5 µm). (E): UV-treated surface (scale bar = 1 µm)

254 and 185 nm. After 15 min of irradiation, the discs and cylinders were left to cool for 5 min before performing surface analysis and surgery.

Surface characterization

Surface morphology and roughness Measurements

The morphologies of the Ti disks surfaces after UV photofunctionalization were examined by scanning electron microscope (SEM; Model JSM-6301F, JEOL, Tokyo, Japan) at 15 kV. Surface roughness on each disc was tested with a profilometer (Surfcom 590A, Seimitsu, Tokyo, Japan). The centerline average roughness value, maximum profile height, and maximum roughness depth were determined by averaging the values for three random regions per surface.

Hydrophilicity assessment

The hydrophilicity of the UV-treated and untreated Ti disks surfaces was assessed by measuring the contact angle of 1.0 µl of ddH$_2$O droplets on the disk using the automatic contact angle meter (CA-X, Kyowa, Saitama, Japan). The hydrophilicity was measured graphically on magnified lateral photographic images of ddH$_2$O droplets.

Surgical Procedures

Sprague-Dawley rats (8-week-old male) were given general anesthesia with 1% to 2% isoflurane inhalation (095-06573, FUJIFILM Wako Pure Chemical Corporation). Their feet were shaved and scrubbed with 10% povidone-iodine solution (ISODINE, Meiji Seika Pharma, Tokyo, Japan). Flat surface of the distal femurs was then selected for implant placement. The implant placement site was set 9 mm from the distal end of the femur to form an implant cavity (Holes were created with a 0.8 mm round burr, enlarged using reamers (#ISO 090 and 100), and the entire implant body was placed.). Cylindrical Ti implants were alternately placed on each side of the femur. The experimental protocol in animals and the surgical procedure followed the guidelines for Animal Care approved by Kanagawa Dental University (No 146, 147).

Biomechanical assessment of bone implant integration

The biomechanical strength at the bone-Ti interface was evaluated by the biomechanical push-in test. Animal surgery and evaluation of bone–Ti integration were performed using an existing procedure[21]. After a 2-week healing period, femur including the cylindrical Ti implant was harvested and the upper surface was embedded in auto-polymerizing resin at the implant level. The implants were loaded vertically downward at a crosshead speed of 1 mm/min in a testing machine (EZ-S, Shimadzu Corporation, Kyoto, Japan) equipped with a 2,000 N load cell and a pushing rod (0.8 mm diameter)[14]. The push-in value was determined by measuring the peak of a load–displacement curve.

Statistics analysis

Seven samples of Ti discs were used for the surface roughness test, five samples of Ti discs were used for measurments contact angle values, and five samples of Ti cylindrical implants were used for biomechanical push-in test for each surface. Statistical analyses were conducted using SPSS statistical software (SPSS Statistics 22.0, IBM Corporation, Armonk, NY, USA). Statistical analysis was performed using analysis of variance (ANOVA). Dunnett's test were used for multiple comparisons. A $p<0.05$ was considered significant.

Results

Microtopography of surfaces before and after UV irradiation

The surfaces of the prepared Ti disks and cylindrical Ti implants were imaged (Fig. 1). As the results of ANOVA showed a significant

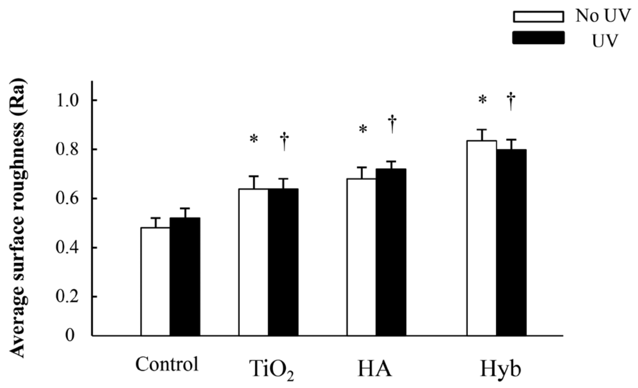

Figure 2. Quantitative measurement of surface roughness of the micropit and nano structures of the different surfaces (n = 7). Comparison of control with modified surface groups. * Significantly different from the control group before UV treatment. † Significantly different from the control group after UV treatment.

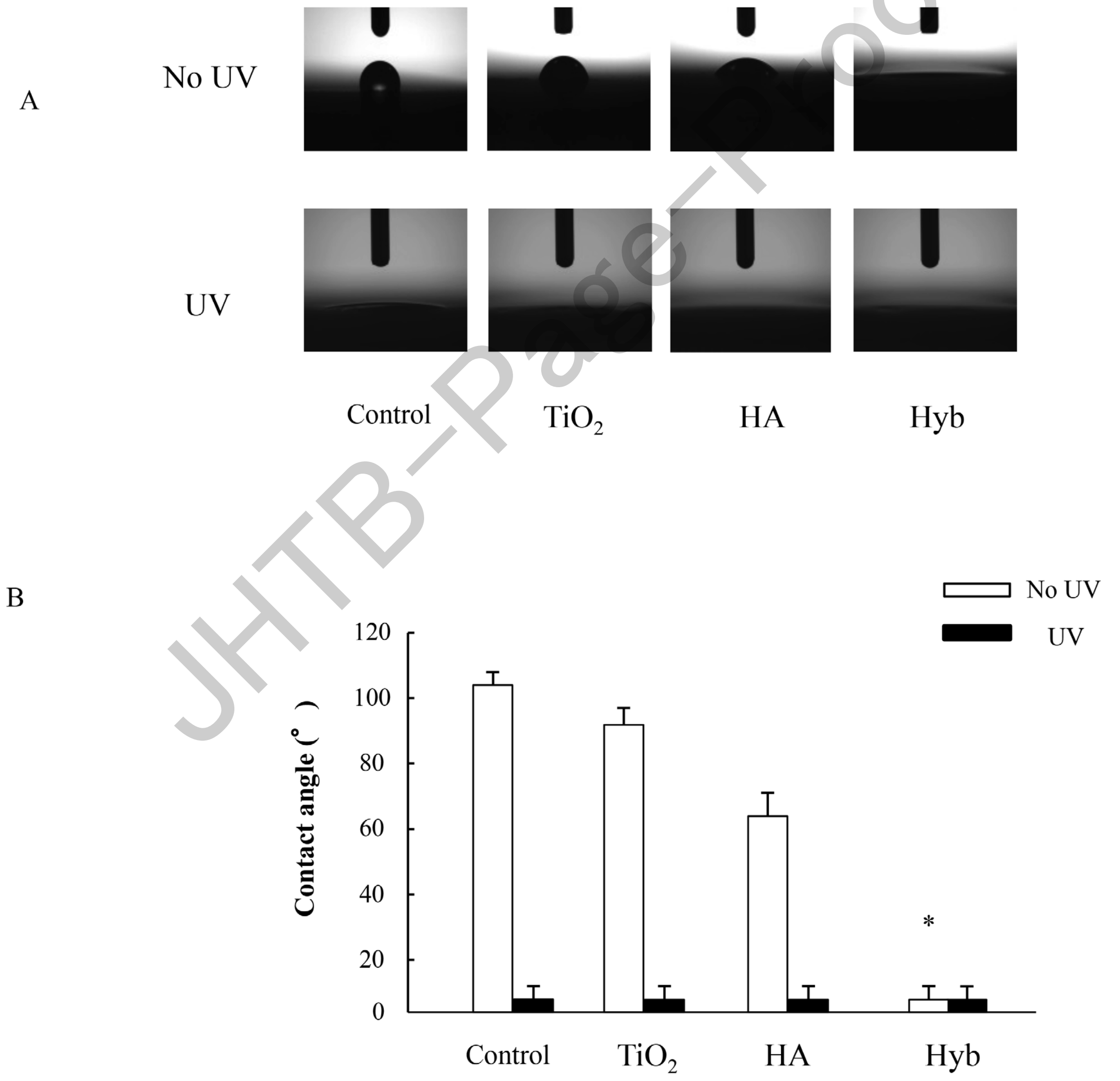

Figure 3. Hydrophilicity assessment of the different surfaces. (A) Image of the droplet from the side of the contact angle to each surface. (B) Contact angle values. Four different surfaces before UV treatment and after UV treatment were tested. Data represent mean ± SD (n = 5). * Significantly different from the control group before UV treatment.

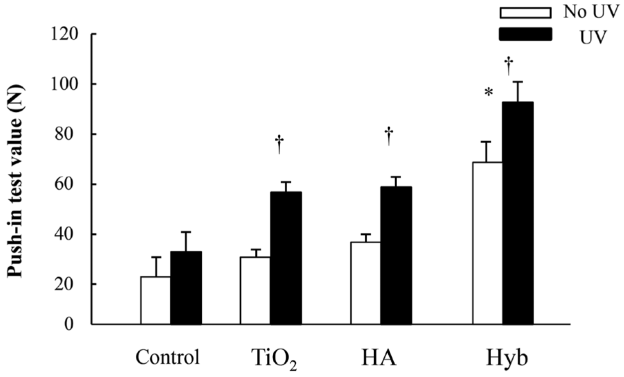

Figure 4. *In vivo* bone integration of the four different implants. Biomechanical strength of bone-implant interface evaluated by the biomechanical push-in test. The values represent mean ± SD (n = 5). * Significantly different from the control group before UV treatment. † Significantly different from the control group after UV treatment.

difference in surface roughness (p<0.01), Dunnett's test was used for multiple comparisons. The TiO_2 and/or HA surfaces showed significant surface roughness (p<0.05; Fig. 2) compared to the control samples with the lowest roughness, as observed for the Hyb surface. The topography of the Hyb surface combined TiO_2 structures (a bio-inert material) and the sediment of HA (a bio-active material). There were no significant differences between the surface roughness values for the UV-treated and untreated surfaces.

Hydrophilicity assessment after UV irradiation

Fig. 3 shows the contact angles and side-view images of super pure water droplets on the UV-treated and untreated surfaces (Fig. 3A, B). As the results of ANOVA showed a significant difference in surface roughness (p<0.01), Dunnett's test was used for multiple comparisons. As a result, the Hyb surface exhibited the lowest contact angle of the untreated surfaces (p<0.05). The contact angle decreased significantly in all surfaces after UV irradiation (p<0.05). However, no significant difference was found between the UV-treated and untreated Hyb surface.

Biomechanical assessment of bone implant integration

The biomechanical strength of the bone–implant interface was evaluated using a biomechanical push-in test (Fig. 4). As the results of ANOVA showed a significant difference in surface roughness (p<0.01), Dunnett's test was used for multiple comparisons. As a result, the push-in values for the implants with UV-treated surfaces at the early healing stage (2 weeks) were significantly higher than those for the implants with untreated surfaces (p<0.05). The push-in for the UV-treated Hyb coated implants was approximately 1.3 times higher than that for the untreated Hyb coated implants and was 2.6 times higher than that for the untreated control sample (acid etched).

Discussion

We have reported previously that an implant coated with a TiO_2/HA hybrid structure containing bioactive and bioinert materials experiences a better biological response than conventional microrough surfaces[14]. This study focused on the biological response to UV irradiated Hyb surfaces. In this experiment, UV irradiation improved the biomechanical strength of the bone-implant interface, the Hyb coated implant, in particular, showed higher bone-implant integration than the microrough or single-target sputtered implants.

UV-treated Ti or biomaterial surface enhances the attachment, spread, proliferation and differentiation of osteoblasts, increases protein adsorption, and promotes the biomechanical strength of bone-Ti interfaces. While these benefits have been widely observed, the mechanism of these improvements remain unclear. There are several surface parameters that influence cellular response to the coated implant surface, including surface morphology and roughness, chemical composition, hydrophilicity, surface energy, and electric charge[8,22-24]. UV photofunctionalization technology improves the hydrophilicity, chemical composition, and electric charge of a surface[16,17,25,26].

It has been established that the characteristics of Ti surfaces can be changed from hydrophobic to highly hydrophilic through UV irradiation. In particular, the microrough surface is produced more rapidly and is more durable than machine treated surfaces[16,17]. In this experiment, UV irradiation did not significantly affect the surface morphology or roughness of the surfaces. However, UV irradiation did increase the hydrophilicity of microrough and single-target sputtered surfaces. Interestingly, the contact angle examination showed that the hydrophilicity of the Hyb surfaces did not change after UV irradiation. This suggests that the Hyb surfaces are already hydrophilic. One of the reasons is presumed to be the influence of fractal structure due to Hyb surface generation. The influence of hydrophilicity on bone-implant integration was not apparent in the results.

Furthermore, it has been reported that UV treated Ti surfaces acquire distinct electrostatic properties[25,26]. TiO_2 surfaces are electronegative, and since proteins and cells are also electronegative, they are mutually repulsed. UV irradiation converts the TiO_2 surface to an electropositive surface, promoting the adhesion of proteins and cell attachment[16]. It was suggested that the change in surface charge improved bone-implant integration. This electrostatic control may replace the effect of hydrophilic conditions.

Ti surfaces exposed to the atmosphere have been found to contain contaminated hydrocarbons[27,28]. Progressive accumulation of organic molecules, particularly those with a carbonyl moiety, on Ti surfaces is considered unavoidable under ambient conditions. UV irradiation reduces the amount of hydrocarbons on the Ti surface, and the hydrocarbon content strongly correlates with the rate of protein adsorption and cell attachment[16.17]. A similar phenomenon may occur in HA on the Hyb surface[29,30]. This may explain the relatively high bone-implant integration results for UV irradiated implants. These results, strongly suggest that the mechanism by which the bone-implant integration of the UV-irradiated Hyb surface was improved was not the effect of hydrophilization but rather the effect of the change in electric charge and the removal of hydrocarbons.

Hyb coated implants demonstrate greater bone-implant integration than conventional microrough surfaces. The fact that the performance of Hyb coated implants can be further enhanced by UV irradiation is a significant discovery.

Within the limitations of this study, the bone-implant interface of Hyb coated implants showed the highest biomechanical strength after UV irradiation. This approach to the modification of the surfaces of implants could contribute to the enhancement of the QOL of patients and diversify the range of available implant treatments, particularly for the elderly.

Conflict of Interests

The authors have declare that no conflict of interests exists.

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